CLAIMS

1.   A method for the detection of cell proliferative disorders characterised in that the CpG methylation status of two or more of the genes ALX4, TPEF, p16/INK4A, APC, caveolin-2, DAPK and TIMP3 and/or their regulatory sequences are determined and from said determined methylation status the presence or absence a colorectal cell proliferative disorder or metastasis therefrom is deduced.

2.   The method according to claim 1 characterised in that said genes are selected from the group consisting ALX4, TPEF and p16/INK4A.

3.   The method according to claim 2 characterised in that said genes consist ALX4, TPEF and p16/INK4A.

4.   A method for the analysis of colorectal cell proliferative disorders characterised in that the CpG methylation status of the gene ALX4 and/or its regulatory sequences are determined and from said determined methylation status the presence or absence of a colorectal cell proliferative disorder is deduced.

5.   A method for the analysis of colorectal cell proliferative disorders characterised in that the CpG methylation status of the gene ALX4 and/or its regulatory sequences and one or more of the genes selected from the group consisting TPEF, p16/INK4A, APC, caveolin-2, DAPK and TIMP3 and/or their regulatory sequences are determined and from said determined methylation status the presence or absence of a colorectal cell proliferative disorder is deduced.

6.   A nucleic acid molecule consisting essentially of a sequence at least 18 bases in length according to one of the sequences taken from the group consisting of SEQ ID NO: 7,8, 15 & 16.

7.   An oligomer, in particular an oligonucleotide or peptide nucleic acid (PNA)-oligomer, for the detection of colon cell proliferative disorders, said oligomer consisting essentially of at least one base sequence having a length of at least 10 nucleotides which hybridises to or is identical to one of the nucleic acid sequences according to SEQ ID NOS:7,8, 15 and 16.

8.   A method according to claims 1 to 5, comprising:

-obtaining, from a subject, a biological sample having subject genomic DNA;

-contacting the genomic DNA, or a fragment thereof, with one reagent or a plurality of reagents for distinguishing between methylated and non methylated CpG dinucleotide sequences within at least two target sequences of the genomic DNA, or fragment thereof, wherein the target sequence comprises, or hybridizes under stringent conditions to, at least 16 contiguous

nucleotides of a sequence taken from the group consisting of SEQ ID NO: 5 to SEQ ID NO: 20 and SEQ ID NO: 48 to SEQ ID NO: 59 said contiguous nucleotides comprising at least one CpG dinucleotide sequence; and

-determining, based at least in part on said distinguishing, the methylation state of at least
5   one target CpG dinucleotide sequence, or an average, or a value reflecting an average methylation state of a plurality of target CpG dinucleotide sequences, whereby detecting, or detecting colon cell proliferative disorders is, at least in part, afforded.

9.   The method of claim 8, wherein distinguishing between methylated and non methylated CpG dinucleotide sequences within the target sequence comprises converting
10   unmethylated cytosine bases within the target sequence to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties.

10.   The method of claim 8, wherein the biological sample is selected from the group consisting of cell lines, histological slides, biopsies, paraffin-embedded tissue, bodily fluids, stool, blood, and combinations thereof.

15   11.   The method of claim 8, wherein distinguishing between methylated and non methylated CpG dinucleotide sequences within the target sequence comprises use of at least one nucleic acid molecule or peptide nucleic acid (PNA) molecule comprising, in each case a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group
20   consisting of SEQ ID NO: 5 to SEQ ID NO: 20 and SEQ ID NO: 48 to SEQ ID NO: 59, and complements thereof.

12.   A method according to claims 1 to 5, comprising:

a.   obtaining, from a subject, a biological sample having subject genomic DNA;

b.   extracting or otherwise isolating the genomic DNA;

25   c.   treating the genomic DNA of b), or a fragment thereof, with one or more reagents to convert cytosine bases that are unmethylated in the 5-position thereof to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties;

d.   contacting the treated genomic DNA, or the treated fragment thereof, with an amplification enzyme and at least two primers comprising, in each case a contiguous sequence
30   of at least 9 nucleotides that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:5 to SEQ ID NO:20 and SEQ ID NOS:48 to SEQ ID NO:59, and complements thereof, wherein the treated genomic DNA or the fragment thereof is either amplified to produce at least one amplificate, or is not amplified; and

e.   determining, based on a presence or absence of, or on a property of said amplificate, the methylation state of at least one CpG dinucleotide of a sequence selected from the group consisting SEQ ID NOS:1 to SEQ ID NO:4 and SEQ ID NOS:45 to SEQ ID NO:47 or an average, or a value reflecting an average methylation state of a plurality of CpG dinucleotides of a sequence selected from the groups consisting of SEQ ID NO:1 to SEQ ID NO:4 and SEQ ID NO:45 to SEQ ID NO:47, whereby at least one of detecting, or detecting and distinguishing between colon cell proliferative disorders is, at least in part, afforded.

13.   The method of claim 12, wherein treating the genomic DNA, or the fragment thereof in c), comprises use of a reagent selected from the group consisting of bisulfite, hydrogen sulfite, disulfite, and combinations thereof.

14.   The method of claim 12, wherein contacting or amplifying in d) comprises use of at least one method selected from the group consisting of: use of a heat-resistant DNA polymerase as the amplification enzyme; use of a polymerase lacking 5'-3' exonuclease activity; use of a polymerase chain reaction (PCR); generation of a amplificate nucleic acid molecule carrying a detectable labels; and combinations thereof.

15.   The method of claim 14, wherein said nucleic acid molecule or peptide nucleic acid molecule is in each case modified at the 5'-end thereof to preclude degradation by an enzyme having 5'-3' exonuclease activity.

16.   The method of claim 14, wherein said nucleic acid molecule or peptide nucleic acid molecule is in each case lacking a 3' hydroxyl group.

17.   The method of claim 16, wherein the amplification enzyme is a polymerase lacking 5'-3' exonuclease activity.

18.   The method of claim 12, wherein determining in e) comprises hybridization of at least one nucleic acid molecule or peptide nucleic acid molecule in each case comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:5 to SEQ ID NO:20 and SEQ ID NOS:48 to SEQ ID NO:59 and complements thereof.

19.   The method of claim 18, further comprising extending at least one such hybridized nucleic acid molecule by at least one nucleotide base.

20.   The method of claim 12, wherein determining in e), comprises sequencing of the amplificate.

21.   The method of claim 12, wherein contacting or amplifying in d), comprises use of methylation-specific primers.

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22.   A kit comprising a bisulfite (= disulfite, hydrogen sulfite) reagent as well as at least one oligomer consisting essentially of at least one base sequence having a length of at least 10 nucleotides which hybridises to or is identical to one of the nucleic acid sequences according to SEQ ID NOS:5 to SEQ ID NO: 0 and SEQ ID NOS:48 to SEQ ID NO:59.

5   23.   The use of a method according to any one of claims 1 to 5 and 8 to 21, a nucleic acid according to claim 6, of an oligonucleotide or PNA-oligomer or a set thereof according to claim 7, of a kit according to Claim 22, in the diagnosis of colon cell proliferative disorders.